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UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

ARIA DIAGNOSTICS, INC. Plaintiff, v. SEQUENOM, INC., Defendant/ Counterclaim-Plaintiff, v. ARIA DIAGNOSTICS, INC., Counterclaim-Defendant, and ISIS INNOVATION LIMITED, Nominal Counterclaim-Defendant.	Case No. 3:11-cv-06391-SI **SUPPLEMENTAL DECLARATION OF DR. MARK I. EVANS IN SUPPORT OF SEQUENOM, INC.'S MOTION FOR PRELIMINARY INJUNCTION** Date: June 22, 2012 Time: 9:00 a.m. Place: Courtroom 10, 19th Floor Judge: Hon. Susan Illston

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I, Mark I. Evans, declare:

I. INTRODUCTION

1. I previously provided a declaration in this case in support of Sequenom's Motion for Preliminary Injunction. I now provide this supplemental declaration in response to the opinions expressed in the declarations and deposition testimony of Dr. Farideh Bischoff and Dr. Eric Fearon submitted on behalf of Ariosa Diagnostics, Inc. ("Ariosa").

2. In this declaration I principally address certain issues raised by Ariosa relating to the validity of the '540 patent. As set out in detail below, it is my opinion that the asserted claims of the '540 patent are not invalid as opined by Dr. Fearon. I also address below the claim constructions proposed by Dr. Bischoff and Ariosa, with which I disagree, and Dr. Bischoff's opinions that the Harmony Prenatal Test does not infringe the '540 patent, with which I also disagree. Just because I may not comment upon a particular opinion, statement, or testimony of Dr. Bischoff or Dr. Fearon, does not mean that I agree with it.

II. SUMMARY OF OPINIONS

3. In my scientific opinion, for the reasons set out in this declaration, claims 1, 2, 8, 19-22, 24 and 25 of the '540 patent are not invalid, contrary to the opinion of Dr. Fearon on behalf of Ariosa.

4. In my scientific opinion, for the reasons set out in this declaration, the '540 claims at issue do not attempt to patent a "law of nature."

5. In my scientific opinion, for the reasons set out in this declaration, the Kazakov article does not anticipate the '540 claims at issue.

6. In my scientific opinion, for the reasons set out in this declaration, the claim constructions for "paternally inherited nucleic acid" and "amplifying" set forth by Dr. Bischoff are not reasonable and not the constructions that the person of ordinary skill in the art would understand from reading the claims of the '540 patent, the specification of the '540 patent, the file history for the '540 patent, and the file history for the continuation application (U.S. Patent Application No. 09/872,063) filed by Drs. Lo and Wainscoat.

7. In my scientific opinion, for the reasons set out in this declaration, I disagree with the non-infringement opinions set forth by Dr. Bischoff. In my scientific opinion, the Ariosa Harmony Prenatal Test infringes the asserted claims of the '540 patent using Sequenom's proposed claim constructions and using Ariosa's proposed claim constructions (without the "negative limitation" on paternally inherited nucleic acid, as discussed further below).

III. QUALIFICATIONS AS AN EXPERT

8. I have worked in the field of prenatal and fetal medicine and diagnosis for over 30 years. A copy of my Curriculum Vitae was attached as Exhibit 1 to my previous declaration. My qualifications as an expert are described in detail in my previous declaration. Evans Decl. ¶¶ 6-15.

IV. MATERIALS REVIEWED, PRIOR CASES, AND RATE

9. In addition to the materials listed in my previous declaration, for this declaration I also reviewed the declarations of Dr. Bischoff, Dr. Fearon and Dr. Stuelpnagel submitted on behalf of Ariosa, the deposition transcripts of Dr. Bischoff and Dr. Fearon, the deposition transcript of Dr. Oliphant of Ariosa, and the file history for the continuation application, together with the other materials cited herein.

10. I am being compensated at my usual hourly consulting fee of $650 per hour for my work on this case. The fee for my time spent testifying at deposition is $4000 for up to three hours with $1200 for each additional hour or part, and the fee for my time spent testifying in court is $14,500 per day. None of my compensation depends upon the outcome of this case.

11. The opinions set forth in this declaration are based on my personal knowledge gained from my professional experience, education, and review of documents and data referred to in this declaration and my previous declaration. I may supplement, refine, or revise my analysis as appropriate, including if additional testimony, documents, or other discovery materials become available. If requested, I will testify at Court hearings and proceedings. I may give a tutorial on the relevant subject matter and use illustrations or other demonstratives to explain and illustrate my opinions. I understand that during the course of these proceedings, additional evidence may

be made available to me. I reserve the right to update my analysis, opinions and conclusions based on any such new information. I also reserve the right to respond to any evidence or arguments that may be offered by Ariosa.

V. LEGAL STANDARDS

12. In reaching my opinions set out in this supplemental declaration, in addition to what is set out in my previous declaration, I have applied the following legal standards.

13. I am informed and understand that the burden of proving that a claim in a patent is invalid is by clear and convincing evidence. I understand that this is a higher burden of proof than the preponderance of the evidence standard (more likely than not) which is the standard I used in my previous declaration for forming my opinions that the claims of the '540 patent are infringed by Ariosa's Harmony Prenatal Test. I am informed and understand that clear and convincing evidence has been described as evidence that produces in one's mind an abiding conviction that the truth of the factual contentions is highly probable.

14. I am informed and understand that anticipation requires that each and every element of the claimed invention be disclosed in a single prior art reference or embodied in a single prior art device. The elements must either be inherent or expressly disclosed. There must be no difference between the claimed invention and the reference disclosure, as viewed by one of ordinary skill in the art. The absence from the reference of any claimed element negates anticipation.

15. I am informed and understand that the prior art disclosure need not be expressly disclosed in order to anticipate, rather, it may be inherently disclosed. To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the reference, and that it would be so recognized by a person of ordinary skill in the art. The mere fact that a certain thing may result from a given set of circumstances is not sufficient. Occasional or incidental results are not inherent.

16. In addition, the prior art reference is not anticipating if the reference is not enabling. The reference must describe the claimed invention sufficiently to have placed a person

of ordinary skill in the art in possession of it. Therefore, an anticipating reference must describe all of the elements of the claims in a single reference, and enable a person of ordinary skill in the art to make and use, without undue experimentation, the claimed invention.

17. Finally, I understand that a claim is invalid as anticipated if the invention was patented or published anywhere, or was in public use (no confidentiality restrictions), on sale, or offered for sale in the United States more than one year prior to the effective date of the patent application.

18. Regarding enablement, I am informed and understand that it is improper to examine the specification to determine if the accused product is enabled. Enablement examines whether the full scope of the claimed invention is enabled, not whether or not the accused product is enabled. Importantly, proper analysis of enablement does not require that the specification describe every possible variant of the claimed invention.

19. I am informed that the legal concept of "undue experimentation" properly relates to the claimed invention, and not to the accused product.

20. I have been asked to assume that to enable a claimed method, the specification need not also enable future improvements to the method.

21. I am informed and understand that issued patent claims are entitled to a presumption of validity, requiring clear and convincing evidence to the contrary. As such, Dr. Fearon was required to present evidence that a substantial question of non-enablement of the claims existed, to be shown at trial by clear and convincing evidence. Such evidence of non-enablement is wholly absent in my opinion.

22. In reaching my opinions in this declaration, I applied the same standards for construing the claims of a patent that I set out and applied in my previous declaration.

23. I have applied the above legal principles in reaching my opinions set out in this declaration.

VI. RESPONSE TO DR. FEARON'S ASSERTION THAT THE '540 PATENT CLAIMS AN UNPATENTABLE "LAW OF NATURE"

24. I disagree with Dr. Fearon's opinion that the asserted claims of the '540 patent "describe no more than a law of nature coupled to well-understood, routine, and conventional steps already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately." Fearon Decl. ¶ 44 and ¶¶ 52-121.

A. There Are Noninfringing Methods to Interrogate cffDNA

25. There are methods to detect cell-free fetal DNA without amplifying the DNA, and without separating maternal blood into a cellular and non-cellular fraction.

26. Attached as Exhibit 1 hereto is a true and correct copy of a product sheet from Helicos Biosciences Corporation, describing their True Single Molecule Sequencing approach, which at page 1 describes their technology as the "first universal genetic analysis platform that does not require amplification. Pursuing a single molecule sequencing strategy simplifies the DNA sample preparation process, avoids PCR-induced bias and errors, simplifies data analysis, tolerates degraded samples." The Helicos machines have been used in the scientific literature for the detection of paternally-inherited cell-free fetal DNA from the cell-free fraction of maternal blood. *See*, *e.g.*, van den Oever, J. M. E. et al., Single Molecule Sequencing of Free DNA from Maternal Plasma for Noninvasive Trisomy 21 Detection, Clin. Chem. 2012; 58:4; 1-8 (a true and correct copy of which is attached hereto as Exhibit 2).

27. Attached as Exhibit 3 hereto is a true and correct copy of Bischoff et al., Detecting fetal DNA from dried maternal blood spots: another step towards broad scale non-invasive prenatal genetic screening and feasible testing, Reproductive BioMedicine Online 2003; 6:3; 349–351. The paper describes a method for using samples of mixed maternal/fetal blood including cell-free fetal DNA that does not involve separation of the cellular and non-cellular components of maternal blood. As described in the paper, "development of a successful blood spot (filter paper) approach provides a simple method for transport and collection, enabling cell-

free fetal DNA to be incorporated into non-invasive screening regimes (maternal serum analysis, fetal nuchal translucency) on a wide scale."

B. The '540 Approach Was In No Way Conventional

28. As I have previously described, the approach in the '540 patent was in no way "conventional." *See*, *e.g.*, Evans Decl. ¶¶ 20-21, 45, 52-53, and 69-71.

VII. RESPONSE TO DR. FEARON'S OPNIONS THAT THE CLAIMS ARE NOT ENABLED

29. I disagree with Dr. Fearon's opinion that "the '540 patent specification does not contain sufficient disclosure to enable a person of skill in the art in 1997 to use the methods in claims 1, 2, 8, 19-22, 24, and 25 to detect aneuploidies in the fetus without undue experimentation." Fearon Decl. ¶¶ 45, 122-145.

30. I have been instructed that, the specification need only enable one of ordinary skill in the art to make and use the full scope of the claimed invention. I am informed that enablement does not require precision, robustness, or commercial success. As I understand it, enablement requires simply that the claims teach a person of ordinary skill in the art how to practice the invention. In my opinion, the claims of the '540 patent are enabled. Experimentation leading to a commercially viable embodiment bears no relation to whether or not the claimed invention was enabled by the specification.

31. It is my understanding that Dr. Fearon confuses the question of enablement of the asserted claims with the use of those claims for a particular purpose. *See* Fearon Decl. ¶ 122. In my opinion the specification taught one of ordinary skill in the art in 1997 how to amplify and detect paternally-inherited fetal nucleic acid from a maternal serum or plasma sample. It is my opinion that none of the claims of the '540 patent required undue experimentation by one of ordinary skill in the art in 1997 in order to amplify and detect paternally-inherited fetal nucleic acid from a maternal serum or plasma sample. With respect to using the '540 invention to detect aneuploidies, Example 2 in the '540 patent clearly enables a person of skill in the art to perform the disclosed method. Dr. Fearon's remarks that "the '540 patent specification offers no insight into how to distinguish one aneuploidy from another using this [Example 2] approach," and "the

'540 patent also offers no insight into how to rule out other potential causes for increased levels of cell-free fetal nucleic acid in maternal serum or plasma" do not affect enablement as I understand it. *See id.* at ¶ 141.

32. Dr. Bischoff relies on the prosecution history and examples in the patent specification to support her opinions as to claim construction. *See* Bischoff Decl. ¶¶ 42-55. Dr. Bischoff agrees that the claims were enabled. In discussing the narrowing of the claims and that they "would be allowable if limited to 'paternally inherited' nucleic acid," Dr. Bischoff states that "[t]his reference to what the PTO found to be enabled refers to the enablement of detecting fetal DNA from the Y chromosome and for detecting the RhD gene from maternal plasma from RhD-negative mothers—circumstances involving paternally inherited sequences known to be received only from the father." Bischoff Decl. ¶¶ 46, 107-108.

33. In addition, there are many examples of scientists who used the claimed methods of the '540 patent to detect Down's syndrome and/or fetal sex, although the methods were not commercialized at the time. *See*, *e.g.*: Lo et al., Increased Fetal DNA Concentrations in the Plasma of Pregnant Women Carrying Fetuses with Trisomy 21, Clin. Chem. 1999: 45:10: 1747-1751 (a true and correct copy of which is attached hereto as Exhibit 4) ("Abnormally high concentrations of circulating fetal DNA are found in a proportion of women carrying fetuses with trisomy 21"); Zhong et. al, Fetal DNA in maternal plasma is elevated in pregnancies with aneuploid fetuses, Prenat. Diagn. 2000; 20:795-798 (a true and correct copy of which is attached hereto as Exhibit 5) ("showing that the levels of fetal DNA are also elevated in pregnancies with other chromosomal aneuploidies … when compared to pregnancies with normal male fetuses"); Alberry et al., Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast, Prenat. Diagn. 2007; 27: 415-418 (a true and correct copy of which is attached hereto as Exhibit 6) ("The Y chromosome DYS14 gene was quantified by real-time quantitative PCR (RT-PCR) for the determination of fetal sex in both plasma and chorionic tissue samples"); and Chiu et al., Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma, PNAS 2008; vol. 105, no.

51: 20458-20463 (a true and correct copy of which is attached hereto as Exhibit 7) ("We have shown that differences in amounts of chr21 DNA sequences in maternal plasma contributed by T21 fetuses compared with euploid fetuses can be unambiguously detected.").

VIII. RESPONSE TO DR. FEARON'S OPINION THAT THE KAZAKOV REFERENCE ANTICIPATES THE ASSERTED CLAIMS OF THE '540 PATENT

34. Dr. Fearon cites a single reference to support his opinion that the claims of the '540 patent are anticipated. Fearon Decl. ¶¶ 151-68. It is my understanding that each and every claim element must be present in the reference in order to qualify as an anticipatory reference.

35. Dr. Fearon admits the "Kazakov" reference does not explicitly teach cell-free fetal DNA. *See* Fearon Decl. ¶ 156. In Dr. Fearon's opinion, the Kazakov reference "teach[es] that that [sic] maternal serum **may** contain cell-free fetal DNA, since that is one of only two possible explanations [the Kazakov reference] provides." *Id* (bold emphasis added).

36. I am informed and understand that an alleged piece of prior art must teach one of ordinary skill in the art each element. I am also informed and understand that an alleged prior art reference which does not explicitly disclose an element, must with certainty inherently disclose that element. It is my opinion that a person of ordinary skill in the art could not state with certainty that amplification and detection of paternally inherited cell-free fetal DNA was disclosed in this reference.

37. It is my opinion that the data in the Kazakov reference are suspect: (1) the actual data shown are essentially unintelligible, as the "size marker" is unresolved (I note that Dr. Fearon also was unable to interpret the data, see below); and (2) the authors admit that they were only able to find one of their fragments of interest in the first trimester—arguing against whatever it was being paternally inherited cell-free fetal DNA because one would expect such DNA to be present throughout pregnancy.

38. Importantly, the authors themselves state that they are unsure which cells may be the source of the "extracellular blood DNA". Fearon Decl. Ex. 13 at p.234. Thus, it is my opinion that one of ordinary skill in the art would not understand the Kazakov reference to be

teaching the detection of cell-free fetal DNA in maternal serum or plasma, much less paternally-inherited cell free fetal DNA—given the uncertainty about the data and conclusions drawn.

39. Dr. Fearon was similarly unable to interpret the data from the Kazakov reference. For example, during his deposition when he was asked about controls, he stated "I stand again to correct my views on that if data to the contrary or that help clarify the situation are provided." Fearon Dep. at 119:23-120:7 (true and correct excerpts from the Deposition of Dr. Eric Fearon are attached hereto as Exhibit 8). Clearly, like myself, Dr. Fearon was uncertain what the data were attempting to show. Dr. Fearon stated "So again, the veracity of these statements that I am making are highly dependent on authentication that these statements are, in fact, what the authors pursued and that my understanding of the translation is correct and so forth." Ex. 8, Fearon Dep. at 122:2-6. Additionally, Dr. Fearon stated at his deposition: "So these are, again, statements that don't have a graphical representation to support what the authors say. So my reading of the paper, not necessarily what others would read, is that the authors have obtained such findings but did not present them in the paper. If provided with a different translation or more data, I might revise that interpretation. But that's my interpretation of the paper which might not be shared with others who read the paper." Ex. 8, Fearon Dep. at 138:18-139:2.

40. In light of the fact that the Kazakov reference in my opinion does not inherently disclose cell-free fetal DNA (especially because part of whatever it discloses disappears after the first trimester) nor paternally-inherited cell-free DNA, this reference does not anticipate any of the claims of the '540 patent.

IX. RESPONSE TO DR. BISCHOFF'S NON-INFRINGEMENT OPINIONS

41. It is still my opinion, as set out in my previous declaration, that Ariosa has infringed at least claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent, which are the claims that Sequenom has asserted in its Motion for Preliminary Injunction. Evans Decl. ¶¶ 86-145. I disagree with Dr. Bischoff's opinions that the Harmony Test does not infringe these claims of the '540 patent.

42. As with my previous declaration, I base my analysis of Ariosa's Harmony Prenatal Test™ on the following papers published on-line in the journals *Prenatal Diagnosis* and *American Journal of Obstetrics and Gynecology* ("AJOG"), in January 2012, namely:

(1) Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy, Sparks AB et al., *Prenatal Diagnosis*, 2012; 32:1–7. (Exhibit 5 to my previous declaration.)

(2) Non-invasive Prenatal Detection and Selective Analysis of Cell-free DNA Obtained from Maternal Blood: Evaluation for Trisomy 21 and Trisomy 18, Sparks AB et al., *American Journal of Obstetrics and Gynecology*, 2012; doi: 10.1016/j.ajog.2012.01.030. (Exhibit 6 to my previous declaration.)

(3) Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18, Ashoor G et al., *American Journal of Obstetrics and Gynecology*, 2012; doi: 10.1016/j.ajog.2012.01.029. (Exhibit 7 to my previous declaration.)

43. I note that Dr. Bischoff has relied upon the same three papers in reaching her opinions. Bischoff Decl. ¶ 71.

A. CONSTRUCTION OF "PATERNALLY INHERITED NUCLEIC ACID"

1. Dr. Bischoff and Ariosa's Construction of "Paternally Inherited Nucleic Acid" Is Not Correct

44. A comparison of Sequenom's and Ariosa's constructions is as follows:

'540 Patent Claim Terms	Sequenom's Construction	Ariosa's Construction
"paternally inherited nucleic acid"	"a nucleic acid that originated from the fetus and which was inherited from the father." See, e.g., Evans Decl. ¶ 93.	"known sequence received only from the father, and not fetal sequence which differs from that of the mother" See, e.g., Bischoff Decl. ¶ 102.

45. As set out in my previous declaration, the person of ordinary skill in the art would understand the term "paternally inherited nucleic acid of fetal origin" to have its ordinary and

customary meaning of "a nucleic acid that originated from the fetus and which was inherited from the father." *See, e.g.*, Evans Decl. ¶¶ 20, 80-85, 90, 93, 99.

46. Dr. Bischoff's and Ariosa's construction of "paternally inherited nucleic acid" has two parts. *See, e.g.*, Bischoff Decl. ¶ 102; Bischoff Dep. 112:9-15 (true and correct excerpts from the Deposition of Dr. Farideh Bischoff are attached hereto as Exhibit 9). The first part is that "paternally inherited nucleic acid" means "a known sequence received only from the father." The second part, which Ariosa refers to as an important "negative limitation," is that "paternally inherited nucleic acid" further means "and not fetal sequence which differs from that of the mother." Ariosa's Opposition to Sequenom's Motion for Preliminary Injunction at 11:6-8.

Ariosa's Construction: "a known sequence received only from the father…"

47. The claim language of the '540 patent does not require that the sequence of the paternally inherited nucleic acid that is amplified must be known in advance. Evans Decl. Ex. 2 at pp.23-26. The claim language simply says "amplifying a paternally inherited nucleic acid from the serum or plasma sample" and "detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample." *Id.* at p.23.

48. The specification of the '540 patent does not require that the sequence of the paternally inherited nucleic acid that is amplified must be known in advance. Evans Decl. Ex. 2. I do not see any express words in the specification stating that the invention is limited in this way. I am informed and understand, that in construing a claim term, it is rarely, if ever, correct to limit the meaning of a claim term to the "preferred embodiments" and examples in a patent specification. Yet Dr. Bischoff's construction of "paternally inherited nucleic acid" limits the meaning of the claims to the "preferred embodiments" and examples in the specification.

49. Dr. Bischoff points to the examples of the '540 patent. Bischoff Decl. ¶¶ 49-55. I agree that the examples disclose the amplification of a paternally inherited nucleic acid from the serum or plasma sample and detection of the presence of a paternally inherited nucleic acid of fetal origin in the sample. But I do not see a requirement that the paternally inherited nucleic acid sequence must be known in advance.

50. In the examples using the Y chromosome (Examples 1, 2, 4, and 5 in the '540 patent), the sequence of the fetal nucleic acid to be amplified is known in advance because specific primers are used to amplify a specific sequence from the Y chromosome. The same is true for the sequence of the Rh.D. gene amplified and detected in Example 3 of the '540 patent. And the same is also true of the Harmony Prenatal Test because, as explained in my previous declaration and below, Ariosa has chosen in advance the specific sequences ("loci") to be amplified using uPCR in their DANSR assay. Evans Decl. ¶¶ 100-103, 105-111.

Ariosa's Construction: "… and not fetal sequence which differs from that of the mother "

51. In support of Ariosa's "negative limitation," Dr. Bischoff relies solely on U.S. Patent Application No. 09/872,063, that was filed by Drs. Lo and Wainscoat. Bischoff Decl. ¶¶ 42, 59-67.

52. I consider Dr. Bischoff's and Ariosa's addition of this "negative limitation" to the meaning of "paternally inherited nucleic acid" to be inappropriate and incorrect because, using this limitation, "paternally inherited nucleic acid" as used in the claims of the '540 patent, would not cover a nucleic acid sequence of the fetus which differs from that of the mother. A person of ordinary skill in the art would not understand "paternally inherited nucleic acid" in this way. I also understand that a claim construction that excludes all of the preferred embodiments or examples in a patent specification is rarely, if ever, correct.

53. Under this negative limitation, "paternally inherited nucleic acid" as used in the '540 patent would *not* include sequences from the Y chromosome. That is because mothers do not have a Y chromosome, and a fetal Y chromosome sequence is a "fetal sequence which differs from that of the mother" – which Ariosa's negative limitation explicitly excludes. However, 4 of the 5 examples in the '540 patent (Examples 1, 2, 4, and 5) describe the amplification of, and the detection of the presence of, sequences on the Y chromosome.

54. Example 3 in the '540 patent describes the amplification (and detection of the presence of) the Rh.D. gene in fetuses, in samples from Rh.D. negative pregnant females. That is, the Rh.D. sequence amplified and detected in Example 3 is a "fetal sequence which differs from

that of the mother," which again is excluded from "paternally inherited nucleic acid" under Dr. Bischoff's and Ariosa's "negative limitation." So Example 3 in the '540 patent would not be covered by the claims of the '540 patent under Dr. Bischoff's and Ariosa's claim construction.

55. In her declaration, Dr. Bischoff discusses the prosecution history of the continuation application. Bischoff Decl. ¶¶ 59-67. In particular, Dr. Bischoff quotes from, and comments about, the "Reply" made by the applicants, Drs. Lo and Wainscoat, which Dr. Bischoff attached as Exhibit 27 to her declaration. *Id.* As quoted by Dr. Bischoff, the applicants stated at page 7 of the Reply that:

> The parent patent 6,258,540 claims in claim 1:
>
> "A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample."
>
> This is just one specific example which illustrates the utility of Applicants' claimed invention. Applicants seek in this continuing application to obtain claims that more fully reflect the generality of the invention. **This is because the term "paternally inherited" does not cover the cases: (a) in which a gene is maternally inherited, yet the nucleic acid is not (in total) the same in the fetus as in the mother, and (b) in which the gene is altered spontaneously, for example, in the egg or sperm, i.e. by what appears to be chance or sporadic mutation.** Also, it is not always necessary to amplify the nucleic acid in the sample in order to detect the fetal DNA.

Bischoff Decl. Ex. 27 (emphasis added).

56. As I have highlighted in bold above, the applicants explicitly stated that "the term 'paternally inherited' does not cover" two specific cases. The first is a **maternally inherited** fetal sequence in which the nucleic acid is not the same in the fetus as in the mother. The second is where the fetal sequence is **altered spontaneously**.

57. This statement by the applicants does *not* say that "paternally inherited" means "a known sequence received only from the father, and not fetal sequence which differs from the mother." Rather, it says that "paternally inherited" does not cover the two specific instances identified.

58. In paragraph 62 of her declaration, Dr. Bischoff refers to a declaration of Dr. Lo submitted during the prosecution of the continuation application. She states "Similarly

suggesting that the '540 patent claims as issued do not cover tests for Down syndrome, Dr. Lo stated…" and she quotes two paragraphs from Dr. Lo's declaration. Dr. Bischoff is clearly mistaken in her analysis of the Lo declaration.

59. The declaration of Dr. Lo does not mention the '540 patent at all. Bischoff Decl. Ex. 28. It is my understanding that the references to "the present invention" in the two paragraphs of the Lo declaration cited by Dr. Bischoff in paragraph 62 of her declaration are directed to the "present invention" as being sought in the continuation application. In her deposition, Dr. Bischoff could not identify any mention of the '540 patent in the Lo declaration. Ex. 9, Bischoff Dep. at 80:10-13.

60. I also note that at the time the continuation application was abandoned, none of the pending claims made any reference to "paternally inherited." *See* Bischoff Decl. Exs. 21-48, and, in particular, Bischoff Decl. Ex. 37, listing pending claims 37-48, at pages 2-5.

B. CONSTRUCTION OF "AMPLIFYING"

1. Dr. Bischoff and Ariosa's Construction of "Amplifying" Is Not Correct

61. A comparison of Sequenom's and Ariosa's constructions is as follows:

'540 Patent Claim Terms	**Sequenom's Construction**	**Ariosa's Construction**
"amplifying"	"increasing the amount by making copies" *See, e.g.*, Evans Decl. ¶ 98.	"increasing the relative concentration of" *See, e.g.*, Bischoff Decl. ¶ 121.

62. As set out in my previous declaration, the person of ordinary skill in the art would understand the term "amplifying" to have its ordinary and customary meaning of "increasing the amount by making copies." Evans Decl. ¶ 98.

63. Dr. Bischoff's and Ariosa's construction contains the limitation that amplification must increase the "relative concentration." Bischoff Decl. ¶ 121; Ex. 9, Bischoff Dep. at 120:5-11. I disagree that this is how the person of ordinary skill in the art would understand "amplifying" as used in the claims of the '540 patent.

64. Claim 2 of the '540 patent, which depends from claim 1, specifies that the amplification step is by PCR, and Ariosa's Harmony Prenatal Test uses amplification by PCR.

65. I do not see any words in the claims of the '540 patent nor in the specification that would require the additional limitation that Dr. Bischoff imposes as to "relative concentration." I also do not see anything in the prosecution file history (or the continuation application file history) to suggest that this "relative concentration" limitation is correct.

66. Dr. Bischoff appears to have relied upon a dictionary definition in determining the meaning of "amplifying." Ex. 9, Bischoff Dep. at 122:1-5. In fact, several other dictionary definitions of amplifying – with which Dr. Bischoff agrees (*Id.* at 128:2-130:5) – support my understanding of the meaning of "amplifying," and do not define amplifying in the more restrictive way as "increasing the relative concentration." See, for example, Exhibit 24, a true and correct excerpt from the Concise Dictionary of Modern Medicine: "DNA amplification: Any method used to increase the copy number of a sequence of DNA." *Id.* at 128:2-13.

67. I also note that in the course of Dr. Bischoff's deposition, she referred to amplification in terms very similar to my understanding. Ex. 9, Bischoff Dep. at 128:2-20 (admitting that "Any method used to increase the copy number of sequence of DNA" is one definition of amplification); Bischoff Dep. at 128:24-130:5 (admitting that "increase in the number of copies of a specific nucleic acid sequence" is another definition of amplification).

C. THE HARMONY TEST INFRINGES THE ASSERTED CLAIMS OF THE '540 PATENT UNDER EITHER SEQUENOM'S CONSTRUCTIONS OR ARIOSA'S CONSTRUCTIONS (EXCLUDING THE "NEGATIVE LIMITATION")

68. For the reasons set out in my previous declaration, the polymorphic assay used in the DANSR process infringes the asserted claims of the '540 patent under Sequenom's proposed constructions. Evans Decl. ¶¶ 86-145.

1. The Harmony Prenatal Test Also Infringes the Asserted Claims of the '540 Patent Under Ariosa's Proposed Constructions (excluding the "negative limitation")

69. As to claims 1, 2, 8, 19-20, 24, and 25, Dr. Bischoff only disagrees with my proposed construction of two terms used in these claims, namely "paternally inherited nucleic

acid" and "amplifying." In addition, Dr. Bischoff also disagrees with my opinion as to the meaning of "prenatal diagnosis" as used in claims 21 and 22 of the '540 patent.

a. The Harmony Prenatal Test Practices The Step Of "Detecting Paternally Inherited Nucleic Acid In The Sample"

70. If "paternally inherited nucleic acid" is construed to mean "known sequence received only from the father," then Ariosa's Harmony Prenatal Test would still infringe the asserted claims of the '540 patent.

71. As set out in my previous declaration (see for example, Evans Decl. at ¶ 110), Ariosa's technical papers on the polymorphic assay used in the DANSR assay explain that:

> To assess fetal fraction, we designed assays against a set of 192 SNP containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. **SNPs were optimized for minor allele frequency in the HapMap3 dataset…**

Evans Decl. Ex. 6 at p.6 (emphasis added). What this means is that Ariosa chose the 192 SNPs because they expected to be able to find paternally inherited sequences that differed from maternal sequences. The Ariosa publication later describes, under the heading "Analysis of polymorphic loci for fetal fraction":

> Informative polymorphic loci were defined as loci where fetal alleles differ from maternal alleles. Because DANSR exhibits allele specificities exceeding 99%, informative loci were readily identified when the fetal allele proportion of a locus was measured to be between 1 and 20%.

Evans Decl. Ex. 6 at p.8.

72. The Ariosa AJOG Publication notes that with respect to Ariosa's polymorphic loci/fetal fraction analysis, "The results correlate well ($R2 > 0.99$) with the weighted average approach presented by Chu and colleagues," (Evans Decl. Ex. 6 at pp.8-9) citing to Chu T, Bunce K, Hogge WA, Peters DG, A novel approach toward the challenge of accurately quantifying fetal DNA in maternal plasma, Prenat. Diagn. 2010; 30:1226-29 (a true and correct copy of which is attached hereto as Exhibit 10). The Chu article says: "We therefore present a novel and highly accurate approach to the measurement of fetal DNA concentrations in maternal plasma based upon the parallel targeted sequencing of multiple **paternally inherited polymorphic biallelic**

markers." *Id*. at p.1 (emphasis added). Likewise, Ariosa is sequencing paternally inherited polymorphic biallelic loci.

73. DNA sequences are highly conserved (almost identical) among humans. One type of genetic variance is the single nucleotide polymorphism ("SNP"), which means a change to only a single base in a sequence. At any particular location ("locus") in the genome, a variant sequence is called an allele. SNPs can have four variants (A, C, G, or T), but for its assay, Ariosa selects "biallelic" loci, where there are typically only two allele variants. See Oliphant Dep. at 35:22-36:10 (true and correct excerpts from the Deposition of Dr. Arnold Oliphant are attached hereto as Exhibit 11). The allele that appears less frequently in the population is called the "minor allele," and Ariosa picks alleles with "high minor allele frequencies" because the higher the minor allele frequency, the more likely any given SNP will be informative for paternally inherited alleles (when the sample has mostly one allele, from the mother, and a small amount—half the fetal fraction—of the allele from the father). *Id.* Accordingly, when Ariosa detects an imbalance at one of the specified polymorphic locus, Ariosa is detecting a paternally inherited nucleic acid from the fetus.

74. Ariosa knows in advance the sequences of the alleles to be amplified and detected at the 192 loci. Evans Decl. Ex. 6 at p.6 ("To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr 1-12, where two middle oligos, differing by one base, were used to query each SNP"). For each polymorphic locus, the Illumina sequencer reports counts of both alleles corresponding to the maternal nucleic acid and the fetal nucleic acid. If there is cell-free fetal nucleic acid in the sample, then the Illumina sequencer will detect the alleles (i.e., the sequence) corresponding to the cell-free fetal nucleic acid at several (if not all) of the chosen polymorphic alleles. That is why Ariosa selected those alleles for the polymorphic assay.

75. There is no doubt that Ariosa's polymorphic assay detects paternally-inherited fetal alleles. First, the whole point is to find "informative" loci; the "information" being sought is the percentage of paternally-inherited DNA in the sample. Ex. 11, Oliphant Dep. at 73:12-75:3, 76:20-77:23, 79:2-7, 81:3-11, 83:11-24. By using biallelic loci (where there are expected to be

two variants in the population), Ariosa calculates percent paternal, which is the same thing as ½ fetal fraction. A conceptual/illustrative explanation for how Ariosa determines fetal fraction is set forth in the charts attached hereto as Exhibit 12, although Ariosa only uses "loci that have a fetal allele proportioned at a locus that is between 1 and 20 percent." Ex. 11, Oliphant Dep. 74:9-15. This concept is also explained in the paper by Chu et al., Ex. 10, noted above.

76. Ariosa has hinted at the possibility that there could be other sources of allelic imbalance in their assay, but in the overwhelming majority of cases, the imbalance is due to the detection of paternally inherited nucleic acid. Spontaneous mutations are much too rare to make up any appreciable portion of the detected nucleic acid in Ariosa's test.

77. Any suggestion that the source of the "differ[ence] from the maternal alleles" (Ex. 11, Oliphant Dep. 140:19-143:10) could be something other than paternal inheritance cannot be taken seriously. While in theory, any given allele could have a spontaneous mutation, the overall mutation rate is in the range of 1-2 x 10^{-8} (that is, 1 in 50-100 million) per base pair per generation. Conrad et al., Variation in genome-wide mutation rates within and between human families, Nature Genetics 2011; 43:7, 712-715 (a true and correct copy of which is attached hereto as Exhibit 13). There are approximately 3 billion ($3x10^9$) base pairs in the human genome, so there are something in the range of 30 mutations in a genome per generation. The likelihood of any particular allele having a mutation is thus in the range of 30 / $3x10^9$, which is 0.00000001%. These numbers are approximate and there are many complicating factors, but it is quite clear with more than 99.99% certainty that Ariosa is detecting paternally inherited nucleic acid. Detection of paternally inherited nucleic acid is the entire basis for the FORTE polymorphic assay

78. For that reason, the Ariosa Harmony Prenatal Test meets the "detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample" element of claim 1 (and the dependent claims) of the '540 patent even under Ariosa's construction of "known sequence received only from the father."

b. The Harmony Prenatal Test Practices The Step Of "Amplifying" Paternally Inherited Nucleic Acid From The Serum Or Plasma Sample"

79. Ariosa's Harmony Prenatal Test, amplifies, even under Dr. Bischoff's proposed construction of "increasing the relative concentration of." As explained in my previous declaration, the DANSR assay uses universal PCR to amplify the ligated product. Evans Decl. ¶¶ 102-103. Ariosa has chosen the universal PCR primers to ensure that the chosen sequences of the polymorphic and non-polymorphic loci are amplified, and that nucleic acids in the sample without the "5' universal amplification tail" and the "3' universal amplification tail" will not be amplified by the uPCR process. *Id.* at ¶¶ 115-117.

80. Accordingly, the relative concentration of the paternally inherited nucleic acids, which correspond to one of the two alleles at the polymorphic loci, is increased relative to other nucleic acids in the sample.

81. Even if "paternally inherited" is interpreted to mean a known sequence, it is clear that the polymorphic assay used in the DANSR process amplifies paternally inherited nucleic acid from the plasma sample and detects paternally inherited nucleic acid in the sample.

82. Regarding claims 21 and 22, I note that Dr. Bischoff did not use the definition of "prenatal diagnosis" specified in the '540 patent at column 2, lines 6-16.

83. In my opinion, the Ariosa Harmony Prenatal Test meets all the limitations of the asserted claims, and therefore infringes the asserted claims of the '540 patent.

X. POTENTIAL HARM TO THE MARKET FOR NONINVASIVE PRENATAL TESTING FROM ARIOSA'S HARMONY PRENATAL TEST

84. I understand that Dr. Bischoff disagrees with my opinion that if Ariosa is permitted to market the Harmony Prenatal Test™ at all, *i.e.*, if it is not enjoined, and if it markets the test to women with low risk of fetal aneuploidy pregnancies without a proper clinical validation, it will significantly harm the market for noninvasive aneuploidy testing. Evans Decl. ¶¶ 147-155.

85. I have reviewed the declaration and deposition testimony of Dr. Bischoff relating to the clinical validation of the Harmony Test, and to statistical analysis pertinent to use of a

diagnostic or screening test. Bischoff Decl. ¶¶ 136-142; Ex. 9, Bischoff Dep. at 183:1-193:1. It is clear from this that Dr. Bischoff does not properly understand statistical analysis applied to testing and clinical trials.

86. When asked if she was an expert on statistical analysis of clinical trials, Dr. Bischoff testified that "I have not claimed to be an expert on statistical analysis." Ex. 9, Bischoff Dep. at 183:1-5.

87. Dr. Bischoff does not understand what "positive predictive value" is. She testified as follows:

> Q What does "positive predictive value" mean?
> A Positive predictive value?
> Q That's right.
> MR. IANCU: Objection; vague, out of context.
> THE WITNESS: In the context of what we're discussing here, positive predictive value is the sensitivity of an assay, the accuracy in detecting the correct marker or target, whatever the test is geared toward or designed to detect or measure.

Ex. 9, Bischoff Dep. at 184:19-185:2.

88. Dr. Bischoff is mistaken. Positive predictive value is *not* the sensitivity of an assay. Furthermore, Dr. Bischoff stated in her declaration that the performance of a test does not decrease as one moves from a high risk to a low risk population because the sensitivity and specificity do not change. Bischoff Decl. ¶ 139. Similarly, Dr. Bischoff incorrectly testified that the positive predictive value of the Harmony Prenatal Test is the same irrespective of the population (high risk or average risk) that it is used for. See Ex. 9, Bischoff Dep. at 185:4-186:20. She explicitly ignored and/or did not understand my testimony that the problem is with the predictive values, not sensitivity and specificity. The low positive predictive value in the low risk population will lead some women to terminate pregnancies based solely on the test when actual diagnostic testing would have shown them to have normal fetuses.

89. In my opinion, Dr. Bischoff's declaration and deposition testimony is an example of the harm that I previously explained will occur from introducing the Harmony Prenatal Test into the low risk market at this time. As I explained, clinicians often do not understand the

difference between sensitivity and positive predictive values. Dr. Bischoff is not a clinician and does not treat patients undergoing screening tests, but undertook in this case to provide an opinion that clinicians would understand the difference. Nonetheless, at her deposition, Dr. Bischoff demonstrated that she did not understand the difference between sensitivity and positive predictive values.

90. Dr. Bischoff is in good company, because that is exactly the mistake made by the majority of clinicians without special training on the subject, which is why there continue to be articles published in leading medical journals about how to teach clinicians to actually understand this. See, for example, Gigerenzer, What are natural frequencies, BMJ 343, 6386 (2011) (a true and correct copy of which is attached hereto as Exhibit 14) (reporting that the vast majority of clinicians surveyed got this wrong) , and Loong, Understanding sensitivity and specificity with the right side of the brain, BMJ 32700716 (2003) (a true and correct copy of which is attached hereto as Exhibit 15) ("I first encountered sensitivity and specificity in medical school. That is, I remember my eyes glazing over on being told that 'sensitivity = TP/TP+FN, where TP is the number of true positives and FN is the number of false negatives.' As a doctor I continued to encounter sensitivity and specificity, and my bewilderment turned to frustration—these seemed such basic concepts; why were they so hard to grasp?").

91. There are numerous harms that likely would result if, as appears to be the case, the Harmony test is marketed for use in the general population of pregnant women. These harms include harm to the mother, the fetus, and the market for non-invasive prenatal tests for fetal aneuploidies. The FDA has expressed the need for monitoring Laboratory Developed Tests (LDTs), of which the Harmony test is one, because use of LDTs which "have ***not been properly validated*** for their intended use put patients at risk…includ[ing]….missed diagnosis, wrong diagnosis, and failure to receive appropriate treatment." Federal Register Volume 75, Issue 116 at 34463 (emphasis added) (a true and correct copy of which is attached hereto as Exhibit 16).

92. Documents produced by Ariosa in connection with the pending motion for preliminary injunction indicate that, [redacted]

[redacted] Exhibit 17. [redacted] *Id.* at AD0020943. [redacted] *Id.* [redacted] *Id.* 93. [redacted] Exhibit 18. [redacted] *Id.* at AD00020938.

94. [redacted] that, if marketed to all pregnant women, the Harmony test should appropriately be categorized as a "high risk" assay, because Ariosa has not validated the Harmony test for commercial use in the general population of pregnant women. In my opinion, marketing of the Harmony test to a ***low-risk*** general patient population would cause harm because many pregnant women who did not need the test nonetheless would invariably elect to have it. Such indiscriminate non-validated testing of low-risk populations is poor medical practice, and can lead to procedures which would not otherwise have been done, are costly, and potentially life threatening to the fetus.

95. Beyond the medical dangers and cost waste, general use of the Harmony test may irreparably harm the non-invasive prenatal testing industry itself should physicians avoid using the screening tests due to the higher numbers of false-positive test results which would result simply due to the significantly higher number of low risk patients tested.

96. The National Society of Genetic Counselors ("NSGC") nicely summarized what responsible physicians, genetic counselors, and test providers know to be true in a position statement adopted on February 18, 2012:

> NSGC does not currently support NIPT as a routine, first-tier aneuploidy screening test in low-risk populations: To date, these technologies have been validated only in pregnancies considered to be at an increased risk for fetal aneuploidy, based on maternal age, family history, or positive serum and/or sonographic screening tests (Palomaki et al., 2011; Palomaki et al., 2012; Bianchi et al., 2012).

NSGC's policy statement dated February 18, 2012 (a true and correct copy of which is attached hereto as Exhibit 19).

97. The NSGC made it clear, and I agree, that non-invasive prenatal LTDs should only be "an option for patients whose pregnancies are considered to be at an increased risk…." *Id*. at p.9. I also agree with the NSGC's assessment that "peer-reviewed data currently supports NIPT only as a screening tool for select populations." *Id.* at p.6.

98. It is my opinion that these tests should be used primarily as secondary screening tests for high-risk patients, and not commercialized as mass use tests in the general population until further studies are performed. While Ariosa's Harmony Prenatal Test boasts of high sensitivity, even a small error rate will be vastly increased in a general population. In addition, Ariosa fails to mention other errors such as accession and processing errors, failed tests, mixed-up results – any number of errors that will geometrically increase if what is currently properly a secondary screen to targeted patients is made available to the general population. There is a very real harm to the public should Ariosa be allowed to market the accused product to the general population of pregnant women.

99. In addition, because the FDA is watching the market for these prenatal tests, the FDA may impose an industry-wide ban or suspension on non-invasive prenatal LDTs for fetal aneuploidies, if Ariosa markets these its Harmony test for general use. Ironically, the high-risk population would then suffer if these useful screening tests were no longer available.

100. Thus, allowing the Harmony test to be marketed to low-risk populations not only places that general population in danger of false positives, but also harms the high-risk population

should the prenatal screen fall out of favor due to such errors, or should the FDA remove them from the market.

101. It is still my opinion that if Ariosa is permitted to market the Harmony Prenatal Test™ at all, *i.e.*, if it is not enjoined, and if it markets the test to women with low risk of fetal aneuploidy pregnancies without a proper clinical validation, it will significantly harm the market for noninvasive aneuploidy testing.

102. I also attach true and correct copies of the following papers cited in paragraph 71 of my previous declaration: Hahn S & Holzgreve W, Prenatal diagnosis using fetal cells and cell-free DNA in maternal blood: what is currently feasible? Clinical Obstetrics and Gynecology, 2002; 45:649-56 (attached hereto as Exhibit 20); Holzgreve W & Hahn S, Fetal cells in cervical mucus and maternal blood, Bailliere's Clinical Obstetrics and Gynaecology, 2000; 14:4:709-22 (attached hereto as Exhibit 21); Pertl B & Bianchi DW, Fetal DNA in Maternal Plasma: Emerging Clinical Applications, Obstetrics and Gynaecology, 2001; 98:483-490 (attached hereto as Exhibit 22); and Uitto J et al., Probing the fetal genome: progress in non-invasive prenatal diagnosis, Trends in Molecular Medicine, 2003; 9:339-343 (attached hereto as Exhibit 23).

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I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct, and that this declaration was executed on June 5, 2012 in Miami Beach, Florida.

/s/ Dr. Mark I. Evans
Dr. Mark. I. Evans

I Michael Malecek, the ECF filer of this document hereby attest that I have on file all holograph signatures for any signatures indicated by a "conformed" signature (/s/) within this efiled document.